

APPLICANT(S): MOORE, Jonni et al.  
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## AMENDMENTS TO THE SPECIFICATION

### In the Specification:

Please replace the paragraph 0028 of Application as published with the following rewritten paragraph:

[0028] In another embodiment, the method further comprises assessing viability of the cell population being evaluated. In one embodiment, cell viability is determined via the use of the vital dye TO-PRO<sup>®</sup>-3.

Please replace the paragraph 0085 of Application as published with the following rewritten paragraph:

[0085] Cell surface marker staining was performed. The following monoclonal antibodies were used: Phycoerythrin (PE) conjugated anti-human CD4, Tricolor - conjugated anti-human CD3, Allophycocyanin (APC) conjugated anti-human CD8 (Caltag, Burlingame, CA). The vital dye TO-PRO<sup>®</sup>-3 (Molecular Probes Inc., Eugene, OR) was used to discriminate live and dead cells.

Please replace the paragraph 0088 of the Application as filed with the following rewritten paragraph:

[0088] Following CFSE (carboxy fluorescein diacetate succinimide ester) labeling, the cells were cultured in 24-well plates at  $2 \times 10^6$  per well in the presence of either media alone, PHA, *Candida*, or either 10 or 100  $\mu$ M of beryllium sulfate. The cells were incubated for seven days at 37°C and 5% CO<sub>2</sub>. Preliminary experiments titrated the optimal dose of CFSE and time of incubation. Surface labeling was performed at the time of harvest using CD3TC, CD4PE and CD8APC in some experiments. TO-PRO<sup>®</sup>-3 was added to define live cells in some experiments.

Please replace the paragraphs 0089 of the Application as filed with the following rewritten paragraph:

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[0089] All data were acquired on a four-color, dual laser FACSCalibur (Becton Dickinson, San Jose, CA). CFSE was measured in FI-1 channel (530/30-nm bandpass filter), CD4PE in the FI-2 channel (585/42-nm bandpass filter), CD3TC in the FI-3-channel (670 -nm longpass filter) using excitation from the 488 nm blue laser and TO-PRO@-3 or CD8APC in FI-4 channel (661/16 bandpass filter) by excitation using the 635 nm red diode laser. Compensation for CFSE in multiparameter flow cytometry is dose dependent and determined empirically.

Please replace the paragraphs 0090 of the Application as filed with the following rewritten paragraph:

[0090] The basic analysis gating strategy was as follows (Figure 1): for analysis of CD3+ T cells alone, an initial region was defined as lymphocytes by light scatter and a broad region was set to include medium to high forward/low side scatter events after stimulation with PHA (Fig. 1A, R1) to include all proliferating lymphocytes. For CD3+ T cells, the R1 data were plotted in a contour plot of CD3TC versus TO-PRO@-3 to identify the live CD3+ T Cells (Fig. 1B, R2). The R2 population was expressed on a contour plot of CD3+CD4+ where a double positive region was defined as R3 ( Fig. 1C). For final analyses, each population was then analyzed for CFSE (FL1) fluorescence, representing proliferation, on a single parameter histogram using a logical gate of either R1\*R2 for CD3+ cells only and R1\*R2\*R3 for CD3+4+cells. In experiments where only data from CD3+ or CD3+4+ cells were collected, a live gate on TO-PRO@-3 negative ("viable") cells was also used in acquisition. When specific CD4+ and CD8+ T cell determination was performed, the viability dye was eliminated. The strategy was similar to that above with light scatter gating (R1) followed by identification of the CD3+ events using a SSC vs CD3+ plot (R2) and CD4+ (R3) and CD8+(R4) identification followed by single parameter histogram analysis of the CFSE fluorescence (for CD4+, R1\*R2\*R3; for CD8, R1\*R2\*R4\*).

Please replace the paragraphs 0094 of the Application as filed with the following rewritten paragraph:

[0094] To verify that detection of cell proliferation by flow cytometry is possible, CFSE-labeled peripheral blood mononuclear cells were cultured for seven days in the presence of culture media alone, PHA, *Candida*, 10 or 100  $\mu$ M beryllium sulfate. After seven days of culture, cells were harvested and surface stained with CD3TC/CD4PE/TO-PRO $\text{\textcircled{3}}$ . PHA and *Candida* were used as positive controls because of their strong T cell stimulation effect. For negative controls, unstimulated cells labeled with CFSE were used. A live gate was used (TO-PRO $\text{\textcircled{3}}$ ) to exclude any dead cells that might have lost CFSE.

Please replace the paragraphs 0095 of the Application as filed with the following rewritten paragraph:

[0095] In the initial series of experiments, the live CD3+ cell population (TO-PRO $\text{\textcircled{3}}$  negative) that were CD4+ high (positive) were gated on and the proportion of cells that had divided (PR) from the histograms was calculated. Flow cytometric analysis of CFSE-labeled lymphocytes revealed a typical pattern of proliferating cells detectable in both the mitogen and beryllium treated cultures demonstrating that the response to beryllium is detectable. Control cultures (unstimulated) exhibited no loss of CFSE intensity (Figure 2, Table 2).

Please replace the paragraphs 0097 of the Application as filed with the following rewritten paragraph:

[0097] In the initial experiments, it was not possible to determine the exact phenotype of the CD3+ / CD4 low cells, although in many cases they may represent CD8+ T cells. Finding a beryllium response in a population of possible CD8+ T cells was unexpected since there is not thought to be a CD8+ response to beryllium *in vitro*. However, in cultured T cells, CD3+ high/CD4+ low T cells may contain not only CD8+ T cells but also CD4+T cells with surface CD4 that has been down regulated. Since in the first series of experiments, gating on live cells , using TO-PRO $\text{\textcircled{3}}$  , was observed not to offer significant advantage over light scatter gating for viability, therefore, TO-PRO $\text{\textcircled{3}}$  was replaced with anti-CD8+ APC. By using

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this four-color flow cytometry staining technique, we could measure the proliferation of CD3+/CD4+ and CD3+/CD8+ cell populations.